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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Docket Number 8717.6006		Type a plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)			
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TITLE OF INVENTION (280 characters max)
INCORPORATION OF ANAEROBIC BACTERIA IN FEED FORMULATION

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ENCLOSED APPLICATION PARTS (check all that apply)	
☒ Specification	11 Pages (including title page)

METHOD OF PAYMENT (check one)		
☐	A check or money order is enclosed to cover the Provisional filing fees	PROVISIONAL FILING FEE
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE [signature] Date April 15, 2002

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☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

Atty. Docket No. 08717.6006

U.S. PROVISIONAL PATENT APPLICATION

OF

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FOR

INCORPORATION OF ANAEROBIC BACTERIA IN FEED FORMULATION

BACKGROUND OF THE INVENTION

[001] Anaerobic bacteria are known to inhabit the intestines of many animals and to play an important role in microenvironments within the intestines. Some positive influence of anaerobic bacteria is in the secretion of digestive enzymes to aid in the degradation of ingested foodstuffs. Alternatively, anaerobes have been shown to colonize the gut lining and prevent colonization by harmful bacteria.

[002] The presence of anaerobic bacteria in agricultural and aquacultural animals is also well documented. Anaerobic bacteria play a large role in ruminant nutrition and proper balance of ruminant microbial populations is critical for optimal animal production. Less is known about aquacultural systems, however the presence of both facultative and obligate anaerobes has been confirmed.

[003] The importance of recombinant proteins for modern medical applications and therapy cannot be overemphasized. Recombinant production methods for bacteria are well developed [Jonasson, 2002 #627]. Many important commercial proteins are produced in bacterial prokaryotic systems, having importance in industry and medical science.

[004] Aquaculture as an industry is rapidly being developed for production of biomass for food (e.g., shrimp and fish farming) [Halvorson, 1999 #534]. The rapid rise of this industry has led to excessive reliance on antibiotics, both for treatment of disease and as prophylactic additions. This is causing the development of bacterial resistance to the available drugs as well as causing pollution of the aquatic environment. The cost of antibiotics is also a burden on the industry.

[005] Agriculture as an industry also has relied heavily on the application of antibiotics for treatment of disease states and prophylatic applications. However, the addition of subclinical amounts of antibiotic as a growth enhancer has the most potential for damaging effect on the development of antibiotic resistance. Since many of the antibiotics used for agriculture are related to antibiotics used for human health, agricultural application of antibiotics could eventually impact human antibiotic therapy.

[006] Thus, there is a need for new methods for maintaining the health of animals. This is especially true for agricultural and aquacultural animals.

SUMMARY OF INVENTION

[007] It is an object of the invention to provide methods for production of a feed containing anaerobic bacteria that provide a benefit to the consuming animal.

[008] It is an object of the invention to provide a composition of a feed containing anaerobic bacteria that provide a benefit to the consuming animal.

[009] These and other objects of the invention are provided by one or more of the following embodiments.

[010] In one embodiment of the invention a method of production of a feed wherein an anaerobic bacterium is added in a viable but stable natural state such as a spore so that the consuming animal is able to consume the anaerobe and have it colonize the intestines of that animal.

[011] In another embodiment of the invention a method of production of a feed wherein an anaerobic bacterium is added in a inviable state such that compounds beneficial to the animal are released into the intestines of that animal.

[012] In one embodiment of the invention a method of production of a feed wherein an anaerobic bacterium is added in a viable but stable natural state such as a spore so that the consuming animal is able to consume the anaerobe and have it colonize the intestines of that animal. At the same time additional probiotic organisms are added in the feed to provide an optimal nutritional state in the animal.

[013] In a further embodiment of the invention the anaerobic organism added to the above embodiments is a recombinant organism.

[014] In a further embodiment of the invention, the composition of the feeds produced in the above embodiments is contemplated.

[015] The inventors have discovered that by addition of viable or inviable anaerobic bacteria to feeds beneficial effects can be provided to the consuming animal.

Description & Examples

Definitions

[016] An "anaerobic bacterium" is an organism that can survive without the presence of molecular oxygen.

[017] A "facultative anaerobic bacterium" is a bacterium that can survive with or without the presence of molecular oxygen.

[018] An "obligate anaerobic bacterium" is a bacterium that can only survive in the absence of molecular oxygen.

[019] A "bacterial spore" is a resistant structure or stage formed by prokaryotes that is capable of germination into viable cells.

[020] A "probiont" is any viable organism that is provided to another organism for the purposes of colonization within that organism with a beneficial effect.

[021] "Viable" is a condition wherein the organism can multiply and sustain itself.

[022] "Non-viable" is a condition wherein the organism is incapable of multiplication or sustaining itself.

Examples

Example 1. Production of an aquacultural feed containing anaerobic bacteria.

[023] Clostridium difficile is grown in anaerobic medium and allowed to sporulate using standard anaerobic methods[Holdeman, 1975 #656]. Spores are collected by centrifugation, washed with phosphate buffered saline (autoclaved and purged with nitrogen or inert gas) and mixed with AquaGrow Enhance (Advanced BioNutrition). This mixing can be done after the AquaGrow Enhance has been spray dried and blended or prior to this blending. The final material is then available for use as a feed for larval fish and/or crustacean culture.

Example 2. Production of a chicken feed containing anaerobic bacterium expressing an antibiotic peptide.

[024] Using standard techniques [Sambrook, 1989 #109], a facultative anaerobe is genetically modified to express any of the following bioactive peptides: cecropin, penaedins, bactenecins, callinectins, myticins, tachyplesins, clavanins, misgurins, pleurocindins, parasins, histones, acidic proteins, and lysozymes. This anaerobe is grown anaerobically to maintain the stability of the peptide in an optimal (non-oxidized form) and the culture harvested by centrifugation. The biomass is mixed with dry yeast and then flash dried either in a spray drier or by vacuum drying. The homogenized powder can then be supplemented into regular chicken feeds to deliver bioactive compound.

Example 3. Anaerobically grown Photobacterium as an addition to aquaculture feed.

[025] Photobacterium damselae subsp. piscicida are grown on blood agar or agar containing hematin under anaerobic conditions between 18 and 22 C. Cells are harvested and killed by heat treatment or by treatment with formaldhyde. Cells are then dried in a spray drier or by use of a vacuum drier. These cells are tested for viability by culturing, if non-vialble then can be added to feeds for use to block adsorption to the gut by live P. damselae subsp. piscicida.

Example 4. Clostridium difficile killed and added as a feed supplement.

[026] Clostridium difficile is grown under anaerobic conditions as previously described[Holdeman, 1975 #656]. The biomass is killed by heat treatment, air-drying or radiation. Cells are then broken by grinding to crack the cells, use of a roller mill or Wiley mill provide sufficient cracking. The most gentle methods used provide the best retention of enzyme activity. The dried cells are incorporated into feed to provide maximal transfer of digestive enzymes to the consuming organism.

References

The following references are cited herein. The entire disclosure of each reference is relied upon and incorporated by reference herein.

1. Halvorson, H. and F. Quezada (1999). "Increasing public involvement in enriching our fish stocks through genetic enhancement." Genet Anal **15**(3-5): 75-84.
2. Holdeman, L. and W. Moore (1975). Anaerobe laboratory manual.
3. Jonasson, P., S. Liljeqvist, et al. (2002). "Genetic design for facilitated production and recovery of recombinant proteins in Escherichia coli." Biotechnol Appl Biochem **35**(Pt 2): 91-105.
4. Sambrook, J., E. Fritsch, et al. (1989). Molecular Cloning: A laboratory manual. Cold Spring Harbor, Cold Spring Harbor Press.

We claim:

1. A method for production of a feed wherein a feed is made comprising an anaerobic bacterium.

2. A method as in claim 1, wherein the feed is an aquaculture feed.

3. A method as in claim 1, wherein the feed is an agricultural feed.

4. A method as in claim 1, wherein the feed comprises additional probiotic elements.

5. A method as in claim 1, wherein the anaerobic bacteria are supplied in spore form.

6. A method as in claim 5, wherein the anaerobes are from the genus Clostridium.

7. A method as in claim 1, wherein the anaerobic bacteria are supplied in a non-viable form.

8. A method as in claim 1, wherein the non-sporulated anaerobic bacteria are supplied in a processed form such that they remain viable in the feed.

9. A method as in claim 1, wherein the anaerobic bacterium is an obligate anaerobe.

10. A method as in claim 1, wherein the anaerobic bacterium is a facultative anaerobe.

11. A method as in claim 1, wherein the anaerobic bacterium is recombinant.

12. A method as in claim 11, wherein the recombinant anaerobic bacterium is expressing a bioactive compound.

13. A method as in claim 11, wherein the recombinant anaerobic bacterium is expressing a protein or peptide.

14. A method as in claim 11, wherein the recombinant anaerobic bacterium is expressing antisense ribonucleic acid.

15. A method as in claim 1, wherein the anaerobe added comprise one of the following genera Clostridium, Fusobacterium, Peptostreptococcus, Bacteriodes, Butyrivibrio, Leptptrichia, Selenomonas, Succinimonas, Succinivibrio, Eubacterium, Lachnospira, Aracnia, Propionibacterium, Actinomyces, Bifidobacterium, Lactobacillus, Treponema, Borrelia, Campylobacter.

16. A feed composition as in claim 1, wherein the feed comprises anaerobic bacteria in a viable formulation.

17. A feed composition as in claim 16, wherein the feed comprises anaerobic bacteria in a non-viable formulation.

18. A feed composition as in claim 17, wherein the feed is an aquaculture feed.

19. A feed composition as in claim 17, wherein the feed is an agricultural feed.

20. A feed composition as in claim 17, wherein the feed comprises additional probiotic elements.

21. A feed composition as in claim 17, wherein the anaerobic bacteria are supplied in spore form.

22. A feed composition as in claim 21, wherein the anaerobes are from the genus Clostridium.

23. A feed composition as in claim 17, wherein the anaerobic bacteria are supplied in a non-viable form.

24. A feed composition as in claim 17, wherein the non-sporulated anaerobic bacteria are supplied in a processed form such that they remain viable in the feed.

25. A feed composition as in claim 17, wherein the anaerobic bacterium is an obligate anaerobe.

26. A feed composition as in claim 17, wherein the anaerobic bacterium is a facultative anaerobe.

27. A feed composition as in claim 17, wherein the anaerobic bacterium is recombinant.

28. A feed composition as in claim 27, wherein the recombinant anaerobic bacterium is expressing a bioactive compound.

29. A feed composition as in claim 27, wherein the recombinant anaerobic bacterium is expressing a protein or peptide.

30. A feed composition as in claim 27, wherein the recombinant anaerobic bacterium is expressing antisense ribonucleic acid.

31. A feed composition as in claim 17, wherein the anaerobe added comprise one of the following genera Clostridium, Fusobacterium, Peptostreptococcus, Bacteriodes, Butyrivibrio, Leptptrichia, Selenomonas, Succinimonas, Succinivibrio, Eubacterium, Lachnospira, Aracnia, Propionibacterium, Actinomyces, Bifidobacterium, Lactobacillus, Treponema, Borrelia, Campylobacter.

ABSTRACT

[027] Incorporation of anaerobic bacteria in a feed such that it delivers a benefit to the consuming animal. Anaerobic bacteria can be viable and colonize the gut to provide this benefit either by displacement of harmful bacteria, secretion of a particular agent (e.g., enzyme, antibiotic or bioactive compound), binding or sequestration of harmful organisms or compounds or beneficial physical effect. Anaerobic bacteria can also be added in a non-viable form wherein the added bacteria provide a benefit to the consuming organism by delivery of preformed compounds such as enzymes, bioactive agents, or polymer. Recombinant anaerobes can be utilized for any of the above purposes. Feeds can contain naked bacteria, spores, treated bacteria such as encapsulated, coated or freeze dried to maintain either viability of stability of active function.